

ORIGINAL ARTICLE

Genetic enhancement of cognition in a kindred with cone-rod dystrophy due to *RIMS1* mutation

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J Med Genet 2007;44:373–380. doi: 10.1136/jmg.2006.047407



Supplementary material is available online at <http://jmg.bmj.com/supplemental>

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Received 1 November 2006
Revised 30 December 2006
Accepted 4 January 2007
Published Online First
19 January 2007

Background: The genetic basis of variation in human cognitive abilities is poorly understood. *RIMS1* encodes a synapse active-zone protein with important roles in the maintenance of normal synaptic function: mice lacking this protein have greatly reduced learning ability and memory function.

Objective: An established paradigm examining the structural and functional effects of mutations in genes expressed in the eye and the brain was used to study a kindred with an inherited retinal dystrophy due to *RIMS1* mutation.

Materials and methods: Neuropsychological tests and high-resolution MRI brain scanning were undertaken in the kindred. In a population cohort, neuropsychological scores were associated with common variation in *RIMS1*. Additionally, *RIMS1* was sequenced in top-scoring individuals. Evolution of *RIMS1* was assessed, and its expression in developing human brain was studied.

Results: Affected individuals showed significantly enhanced cognitive abilities across a range of domains. Analysis suggests that factors other than *RIMS1* mutation were unlikely to explain enhanced cognition. No association with common variation and verbal IQ was found in the population cohort, and no other mutations in *RIMS1* were detected in the highest scoring individuals from this cohort. *RIMS1* protein is expressed in developing human brain, but *RIMS1* does not seem to have been subjected to accelerated evolution in man.

Conclusions: A possible role for *RIMS1* in the enhancement of cognitive function at least in this kindred is suggested. Although further work is clearly required to explore these findings before a role for *RIMS1* in human cognition can be formally accepted, the findings suggest that genetic mutation may enhance human cognition in some cases.

A genetic contribution to variation in human intelligence is well established, but the identities of the genes responsible remain elusive. Many mutations are associated with impaired cognition:¹ no definite genetic causes of enhanced cognition are established,² and there are no known cognition-enhancing “gain-of-function” mutations in genes otherwise associated with cognitive impairment. Therapeutic possibilities deriving from the discovery of any such genes or variants are potentially important: cognitive decline reduces the quality of life,³ and low intelligence test scores are associated with increased morbidity and shorter life-span.⁴ Accelerated evolution of genes subserving neurodevelopment figures in molecular explanations of the advance of the human nervous system: many of the identified genes regulate brain size and behaviour, some encoding critical synaptic proteins.⁵

To identify genes influencing human brain development and function, including cognitive function, we use a paradigm evaluating cerebral structure and function in individuals with known mutations in genes co-expressed in the lineage-sharing eye and brain, ascertained by their obvious ocular phenotype, but in whom a neurological phenotype was not fully appreciated. Using this paradigm, we demonstrated roles for the genes *PAX6*, *PITX2*, *SOX2* and *OTX2* in human brain development, cognitive function and memory.^{6–11}

We now report on the functional and structural effects of mutation in the eye- and brain-expressed gene *RIMS1*, through

the study of individuals from a family already reported to have retinal dystrophy caused by *RIMS1* mutation.^{12–13} To our knowledge, this is the only family so far reported with such a mutation: the eye phenotype is homogeneous in the family, and has been documented in detail.¹³ The orthologous murine *Rim1a* encodes a synaptic active-zone protein necessary for preserving the normal probability of synaptic neurotransmitter release and for long-term presynaptic potentiation.^{14–15} *Rim1a* is also expressed in retinal ribbon synapses.¹⁶ Mice lacking *Rim1a* protein show severely impaired learning and memory.¹⁷ In our kindred, *RIMS1* mutation (Arg844His) causes a late-onset dominantly inherited cone-rod dystrophy (CORD7; OMIM 603649), leading to varying degrees of visual loss starting from the third decade onwards.¹³ Because *RIMS1/Rim1a* is also expressed in the brain, we hypothesised that this *RIMS1* mutation would produce a structural and functional neurological phenotype.

MATERIALS AND METHODS

Ethics

The study of the kindred was approved by the Research Ethics Committees of the Institute of Neurology, National Hospital for Neurology and Neurosurgery and Moorfields Eye Hospital, and

Abbreviations: LBC, Lothian birth cohort; LOD, logarithm of odd; SNP, single-nucleotide polymorphism; VIQ, verbal IQ

The Lothian Birth Cohort study was approved by the Lothian Research Ethics Committee. All participating individuals provided written informed consent. Human embryonic/fetal material was studied by the Human Developmental Biology Resource at the Institute of Child Health with full ethics approval.

Cognitive tests applied to kindred

The neuropsychological tests used had been used in an earlier investigation of individuals heterozygous for *PAX6* gene mutations.⁷ All the standardised tests selected always require verbal interaction only, accounting for variable vision impairments: subjects with preserved vision (both mutation carriers and unaffected kin) were thus not disadvantaged. Details of the tests used are given in the supplementary material (available online at <http://jmg.bmj.com/supplemental>). Intellectual level, executive and memory functions were tested.

Brain imaging

Details of the high-resolution MRI are provided in the supplementary material (available online at <http://jmg.bmj.com/supplemental>).

Population genetics

Subjects

All subjects were selected from the Lothian birth cohort of 1921 (LBC1921) who are survivors from the Scottish Mental Survey of 1932.¹⁸ On 1 June 1932, a valid mental ability test, a version of the Moray House Test No 12, was administered to almost all Scottish children who were born in 1921 and attending school that day ($n = 87\,498$). The mean (SD) age at re-test was 79.1 (0.6) years. The following inclusion criteria were adopted for each subject: (1) Moray House Test score at age 11 years had to be available; (2) there had to be no history of dementia; (3) Mini-Mental State Examination score was ≥ 24 ; and (4) genotyping had to be successful for *RIMS1*. This gave a total of 469 subjects (278 women).

Cognitive testing

Details are provided in the supplementary material (available online at <http://jmg.bmj.com/supplemental>): Mini-Mental State Examination, Moray House Test, Raven's Standard Progressive Matrices, verbal fluency, logical memory and the National Adult Reading Test were measured. The g factor, as a measure of general intelligence, was created by principal component analyses of some of these measures: a single component accounted for 53.5% of the total variance.

Tag selection

Genotypes of *RIMS1* single-nucleotide polymorphisms (SNPs) with a minor allele frequency of at least 0.05 were downloaded for CEPH trios from the HapMap database, across a 750 kb region of DNA comprising the *RIMS1* gene, with 100 kb upstream and downstream (242 SNPs). The region was divided into seven sections of high linkage disequilibrium using the pairwise D' plot. Haplotypes were inferred for each section (TagIT: <http://www.genome.duke.edu/resources/computation/software>). These haplotypes were then used, within TagIT, to select tagging SNPs for each section, ensuring that the average-locus haplotype r^2 value of each section within the gene was ≥ 0.8 . Genotyping and statistical analysis were carried out as detailed in the supplementary methods (available online at <http://jmg.bmj.com/supplemental>).

RIMS1 sequencing in LBC1921

PCR mix composition, primer sequences, cycling protocols and sequencing details are available on request.

RIMS1 expression studies

RIMS1 mRNA expression was characterised in random-bred CD1 adult mouse brain and eye. The distribution of *RIMS1* mRNA was studied in the human brain and eye at the Carnegie stage 21 and the fetal stage F1. Riboprobe generation and in situ hybridisation are detailed in the supplementary material (available online at <http://jmg.bmj.com/supplemental>).

RIMS1 evolution

Sequencing

Human, chimpanzee, rat and mouse sequences were obtained from GenBank (acc nos NM_014989, XM_527433, NM_052829 and NM_053270, respectively). Sequences were aligned using BioEdit, and DNA mutations were identified. *RIMS1* sequences for macaque and squirrel monkey were sequenced from genomic DNA. PCR primers were designed using the human *RIMS1* genomic sequence as a template, and the presence of PCR product was confirmed by gel electrophoresis. Sequencing was conducted using the BigDye terminator kit and sequences read on an ABI 3700.

Calculation of K_A/K_S

K_A and K_S were determined according to the following definitions:

K_A —number of non-synonymous substitutions per non-synonymous site

K_S —number of synonymous substitutions per synonymous site

Sequences were aligned using BioEdit, and only portions for which sequences were available for both species were included in the calculations. The amino acid content was ascertained for both species and the average number of synonymous and non-synonymous sites was calculated according to the following:

Non-synonymous sites = non-degenerate sites + 2/3 two-fold degenerates.

Synonymous sites = four-fold degenerates + 1/3 two-fold degenerates.

The average number of synonymous and non-synonymous sites was used for the calculation of K_A and K_S . The significance of K_A/K_S between primates and rodents was assessed using a simple χ^2 evaluation.

RESULTS

Cognitive function is enhanced in individuals with *RIMS1* mutation

The pedigree is illustrated in fig 1. Eight (of nine total) affected and seven (of nine total) unaffected adults were assessed. The verbal IQ (VIQ) is a composite measure generated from a range of tests. VIQ was pro-rated from the Wechsler Adult Intelligence Scale (revised) Vocabulary, Digit Span and Similarities subtests.¹⁹ VIQs in affected individuals (table 1) were all above average, with quotients ranging from the 75th (high average ability) to the 99th centile (superior ability). Centile levels for subjects II:2 and II:6 are probably underestimates, as normative data are available up to 75 years only. Some pro-rated VIQ scores, for example 150 in subject III:1, reflect a ceiling effect. On measures of memory and executive skills, performance levels were more variable, with some scores falling below average. Normative data available for these measures were based on smaller sample sizes than for VIQ and, with the exception of semantic fluency, were not available for older age bands.

The identification of *RIMS1* (and its mutation) as a causal factor in this kindred might usually be undertaken using linkage analysis, both for the previously reported retinal phenotype (maximum logarithm of odd (LOD) score 3.61 at $\theta = 0$) and for the phenotype being considered here, VIQ. For

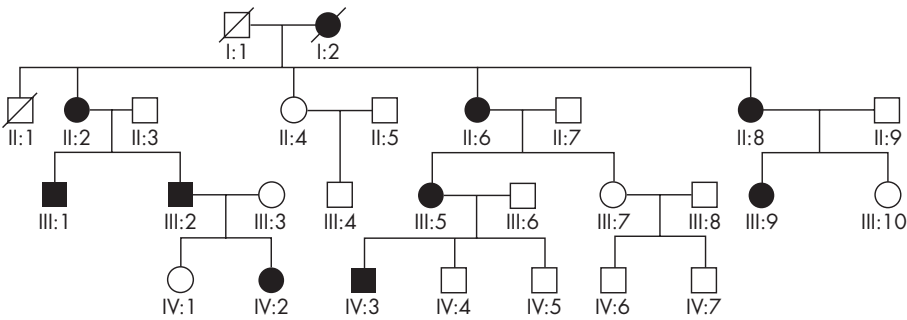


Figure 1 The kindred studied. Filled symbols represent affected individuals, unfilled symbols represent unaffected individuals. Note that the kindred is an extension of that presented by Johnson *et al*¹² and Kelsell *et al*,²⁰ reordered by age of generation II (and thus does not seem to be identical to previous presentations).

VIQ, fewer informative members were tested: using a high IQ phenotype classified as >1 SD (15 verbal IQ points) above the mean) to calculate linkage gives a maximum LOD value of 1.92 at a recombination fraction (θ) of 0.2, which is highly indicative of linkage. This analysis, however, imposes a somewhat arbitrary interpretation of the VIQ phenotype, and does not allow to be taken into account other cognitive information available.

Fortunately, the process of Mendelian inheritance allows a further robust test of the causality of the Arg844His mutation to be performed, avoiding the usual problems of confounding from other variables that might aggregate in a familial fashion but might be unlinked to the mutation. To test the relevance of the mutation, affected and unaffected first-degree sibs in each generation (II:2, II:6, II:8 vs II:4; III:5 vs III:7; IV:3 vs IV:4, IV:5) underwent identical cognitive testing (table 1). The mean age of affected members (53.8 years) was higher than that of unaffected members (41.1 years), acting conservatively against any superior cognitive performance in the affected group, given the known effect of age.²¹ We tested the significance of the association between affected status and every measured cognitive phenotype following a randomisation approach that accounts for the kindred structure.²² *RIMS1* genotypes are randomised across the pedigree, following Mendelian inheritance, while keeping the phenotype values of each individual fixed. The null hypothesis therefore allows for the possibility of familial effects on phenotypes that are unlinked to *RIMS1*, and any significant departure from the null hypothesis can only be ascribed to an effect linked to *RIMS1*. The 11 measured

phenotypes were vocabulary (subcomponent of VIQ), digit span (subcomponent of VIQ), similarities (subcomponent of VIQ), phonemic fluency, cognitive estimates, immediate story recall, delayed story recall, immediate verbal learning, delayed verbal learning, semantic fluency and Hayling test.

The perfect observed association between the affected status and *RIMS1* mutation allowed us to infer a dominant Mendelian disease model, and also to infer the genotypes of all founders and marry-ins. We simulated genotypes, and then the affected status of all individuals in the pedigree based on Mendelian inheritance from the known founders, and for each normalised phenotype we calculated the difference in means between the affected and non-affected individuals (keeping phenotype values fixed among individuals). To address multiple testing issues, we generated a single score for the overall difference between the affected and non-affected groups. We first normalised all phenotypes to a common variance scale, dividing by the standard deviations estimated from a disease-control group of 15 visually impaired individuals with mutation in *PAX6*,⁷ so all phenotypes had equal weight. We then used the Euclidean distance of the 11 difference-in-means values. To control for possible ascertainment bias, we further conditioned on the observed number of the affected and non-affected individuals in the pedigree by rejecting any pedigrees from the Monte Carlo process that did not match this. We carried out 10 000 simulations under these conditions.

The single score for the overall difference in all mean phenotype values between the affected and non-affected subjects was significant ($p = 0.006$). For individual phenotypes,

Table 1 Scores and centile performance (age corrected) for verbal IQ, memory and executive skills tests													
Subject	Age (years)	VIQ	Vocabulary	Similarities	Digit span	Verbal recall immediate	Verbal recall %	Verbal learning trials	Verbal learning: delay	Fluency phonemic "s"	Fluency animals	Hayling total score	Cognitive estimates
Affected													
II:2	82	117 (84th)	14	12	15	26 (25th)	92 (25th)	31 (10th)	6 (25th)	25	19 (75th)	13 (10th)	1
II:6	75	117 (84th)	14	13	13	24 (25th)	83 (25th)	40 (25th)	9 (50th)	19	18 (75th)	14 (10th)	3
II:8	60	113 (75th)	11	10	17	29 (25th)	100 (75th)	50 (50th)	9 (25th)	25	17 (50th)	19 (50th)	1
III:1	53	150 (99th)	18	16	18	41 (75th)	90 (25th)	55 (50th)	15 (90th)	30	30 (90th)	20 (75th)	0
III:2	49	146 (99th)	16	16	16	29 (25th)	79 (10th)	64 (90th)	12 (50th)	28	36 (90th)	22 (95th)	0
III:5	54	129 (98th)	14	14	15	38 (50th)	95 (50th)	58 (50th)	13 (75th)	30	31 (90th)	19 (50th)	2
IV:2	19	112 (75th)	10	12	11	27 (25th)	107 (90th)	62 (75th)	13 (75th)	16	21 (50th)	19 (50th)	6
IV:3	38	134 (98th)	12	15	14	52 (90th)	96 (75th)	54 (50th)	13 (75th)	18	21 (50th)	21 (90th)	2
Unaffected													
II:4	81	102 (50th)	10	11	11	25 (25th)	80 (25th)	36 (10th)	7 (25th)	22	18 (75th)	14 (10th)	5
III:4	57	92 (25th)	8	10	10	13 (<1st)	77 (10th)	33 (<1st)	5 (<1st)	15	18 (25th)	17 (50th)	4
III:7	48	113 (75th)	9	11	11	28 (25th)	86 (25th)	51 (50th)	12 (50th)	16	25 (75th)	17 (50th)	3
IV:4	30	81 (9th)	6	7	7	28 (25th)	94 (50th)	41 (<1st)	9 (10th)	15	20 (50th)	15 (25th)	4
IV:5	24	103 (50th)	9	1	11	38 (50th)	95 (50th)	55 (25th)	11 (25th)	17	23 (50th)	12 (5th)	7
IV:6	26	101 (50th)	9	11	11	20 (<1st)	95 (50th)	36 (<1st)	9 (10th)	18	26 (75th)	18 (50th)	5
IV:7	22	103 (50th)	7	12	12	14 (<1st)	71 (<1st)	49 (25th)	10 (25th)	16	20 (50th)	16 (25th)	5
The genealogical relationships among subjects are shown in fig 1.													

Table 2 Correlation coefficients of phenotypes in disease-control (*PAX6*) sample

	VIQ	Vocabulary	Digit span	Similarities	Phonemic fluency	Cognitive estimates	Story recall immediate	Story recall delayed	Verbal learning immediate	Verbal learning delayed	Semantic fluency	Hayling score
VIQ	1	*	*	*	0.44	-0.25	0.38	0.48	0.58	0.42	0.41	0.02
Vocabulary		1	0.55	0.45	0.36	-0.27	0.33	0.50	0.43	0.35	0.42	0.03
Digit span			1	0.04	0.36	-0.12	-0.06	0.08	0.50	0.18	0.09	-0.35
Similarities				1	0.32	0.02	0.50	0.49	0.31	0.31	0.43	0.58
Phonemic fluency					1	-0.03	0.52	0.66	0.44	0.44	0.69	0.13
Cognitive estimate						1.00	-0.06	0.04	0.26	0.20	0.00	-0.20
Story recall immediate							1.00	0.90	0.18	0.45	0.53	-0.04
Story recall delayed								1.00	0.30	0.56	0.64	-0.08
Verbal learning immediate									1.00	0.76	0.47	0.09
Verbal learning delay										1.00	0.58	0.02
Semantic fluency											1.00	0.24
Hayling score												1.00

*Part-whole correlation.

VIQ, verbal IQ.

all except those for immediate story recall, delayed story recall and semantic fluency had *p* values <5%: vocabulary (*p* = 0.014), digit span (*p* = 0.020), similarities (*p* = 0.039), phonemic fluency (*p* = 0.050), cognitive estimates (*p* = 0.012), immediate story recall (*p* = 0.112), delayed story recall (*p* = 0.087), immediate verbal learning (*p* = 0.048), delayed verbal learning (*p* = 0.042), semantic fluency (*p* = 0.220) and Hayling test (*p* = 0.010). As expected, given that the three subphenotypes of VIQ were all significant, when we separately tested VIQ itself this was also significant (*p* = 0.014).

We determined to what extent the coincidence of these associations is due to the natural correlations among these phenotypes. Table 2 presents the correlation coefficients of the 11 phenotypes and VIQ, in the disease-control sample of 15 individuals with *PAX6* mutation.⁷ Given that the three subphenotypes of VIQ were all significant, when we separately tested VIQ itself, this was also found to be significant (*p* = 0.014): although this particular result reflects a part-whole correlation, it is of note that all the 11 cognitive phenotypes did not naturally correlate with each other, as assessed in the disease-control group.⁷ Phonemic fluency, verbal learning and semantic fluency were highly correlated with VIQ, and it is unclear whether the significant results observed in some of these variables were simply due to correlation with VIQ. However, the phenotypes of cognitive estimates and Hayling test were poorly correlated with VIQ, the other variables and each other, possibly suggesting that multiple facets of mental processes were being affected independently by the mutation.

There was no history or genetic evidence for inbreeding (number of markers scored = 16, heterozygous average for 14 members of kindred = 11.6 (72%); equivalent scores for five "married-ins" = 11.4 (71%); data from ref 22, omitting IV:4–7).

To address the specific, but unlikely,²³ possibility that visual impairment might enhance function in other domains, we compared identical measures from the disease-control cohort of 15 individuals (mean age 36 years, range 17–54 years; eight women), all of whom had congenitally symptomatic *PAX6* mutations.⁷ The *PAX6* subjects had earlier onset of visual symptoms and more severe central visual impairment at the time of cognitive testing than the *RIMS1* subjects (supplementary table 1 available online at <http://jmg.bmj.com/supplemental>). The mean VIQ of the *PAX6* subjects was 103. The *PAX6* subjects as a

group do not differ from the *RIMS1*-unaffected individuals: cognitive measures for both groups fall within the normal ranges (table 3). Despite their more severe and earlier-onset visual impairment, the *PAX6*-affected individuals performed significantly less well than the *RIMS1*-affected individuals (VIQ, Mann-Whitney U test *p* < 0.0005). In addition, in three of eight affected *RIMS1* individuals, visual acuity was normal (6/6) or near normal (6/9) at the time of cognitive assessment.

Brain structure in individuals with *RIMS1* mutation

In seven affected members of the kindred, high-resolution brain MRI was obtained. In two, a mother (II:2) and son (III:1), brain abnormalities were apparent on inspection (fig 2A, B). Cortical grey matter was preserved around widened cerebrospinal fluid spaces: these changes would be compatible with a bilateral parasagittal polymicrogyric malformation.

RIMS1 genetic variation in other cone-rod dystrophy patients and a population cohort

We sequenced the mutation-containing *RIMS1* exon 13 in a panel of 50 unrelated individuals with autosomal dominant cone-rod dystrophy, but did not detect any mutations. Common variation in *RIMS1* (uncorrelated with the rare Arg844His mutation) did not influence cognitive function in LBC1921, for either genotype or haplotype (see supplementary tables 2 and 3 available online at <http://jmg.bmj.com/supplemental>). To determine if mutation in *RIMS1* might account for the upper extreme of performance on cognitive measures, the entire *RIMS1* gene was sequenced in the top-scoring 5% of the LBC (24 individuals). Only one, previously unreported, SNP was found, in residue 592, exon 9: it was synonymous, conserving a glutamic acid residue in an unremarkable region of the gene.

RIMS1 brain expression in human fetal and adult brain

In situ hybridisation with an antisense *RIMS1* probe in fetal stage 1 human embryos revealed *RIMS1* expression in the outer plexiform layer of the developing retina, but not in the optic nerve, as expected (figs 3A–C, F). Cerebral expression was seen in the ventricular zone, developing cortical plate, thalami and hippocampal anlage (fig 3C–E, G). Telencephalic *RIMS1* hybridisation signal was detectable from 57 days after-conception onwards, but not before. In adult human hippocampi from patients who had undergone therapeutic resective surgery for

Table 3 *RIMS1* and *PAX6* group cognitive test scores

Cognitive measures	<i>RIMS1</i> -affected mean (SD)	<i>RIMS1</i> -unaffected mean (SD)	<i>PAX6</i> -affected mean (SD)
Age	53.8 (19.9)	41.2 (22)	36.9 (10.2)
Verbal IQ	127.3 (15)	99.3 (10.1)	104.3 (12.6)*
Vocabulary	13.6 (2.6)	8.3 (1.4)	10.9 (2.6)*
Digit span	14.9 (2.2)	10.4 (1.6)	8.4 (3.0)*
Similarities	13.5 (2.1)	10.6 (1.9)	12.4 (2.5)
Story recall			
Immediate	33.3 (9.6)	23.7 (8.8)	37.7 (8.2)
Delayed	31.0 (9.7)	20.9 (9.3)	33.8 (8.7)
List learning			
Learning trials (max = 75)	51.8 (11.2)	43.0 (8.6)	49.1 (10.1)
Recall (max = 15)	11.3 (3.0)	9.0 (2.4)	10.8 (3.2)
Verbal fluency			
Letters	23.9 (5.5)	17.0 (2.5)	17.1 (7.9)
Animals	24.1 (7.1)	21.4 (3.3)	20.2 (7.2)
Hayling test			
Total score	18.5 (3.3)	15.6 (2.1)	14.9 (3.9)
Cognitive estimates error score†	1.5 (1.2)	4.7 (1.3)	3.9 (2.2)

*Mann–Whitney U test (*RIMS1*-affected vs *PAX6*-affected), actual p values: p <0.005.
†Higher score equates to a poorer performance.

refractory temporal lobe epilepsy due to hippocampal sclerosis, *RIMS1* expression was observed at least in the granule cell layer and CA1 (fig 3H): in the absence of other suitable adult brain material, expression elsewhere cannot be excluded.

RIMS1 evolution

The value of the ratio of non-synonymous (K_A) to synonymous (K_S) nucleotide substitution is a measure of selection pressure on a gene: overwhelmingly, non-synonymous alterations are deleterious, and subject to negative selection. Rarely, non-synonymous changes improve protein function and are subject to positive selection, with an elevated K_A/K_S . To determine whether *RIMS1* has been subject to positive selection, we estimated *RIMS1* K_A/K_S for humans compared with chimpanzee and macaque, considered against rate change in a rodent divergence (rat–mouse) following Dorus *et al.*⁹ We were unable to demonstrate accelerated evolution of *RIMS1* in the primate lineage (see supplementary material available online at <http://jmg.bmj.com/supplemental>).

DISCUSSION

Few individual genetic factors underlying the heritability of human intelligence are known.² Mutations in many genes, inherited in Mendelian fashion, are associated with reduced intelligence,¹ often associated with other problems. Notably, recent variation in two such genes (*MCPH1* and *ASPM*) has been shown to be subject to strong positive selection, indicating ongoing adaptive evolution of the human brain^{24 25} and emphasising the importance of population genetic analyses of genes mutations in which affect human cognition. Quantitative trait analyses in populations with learning disabilities also suggest that genes with Mendelian effects, when mutated, are broadly candidates to influence normal variation in learning abilities in the general population.²⁶ To our knowledge, no human genetic mutations have yet been identified that enhance cognitive function.

We show that a mutation in *RIMS1* is associated, in the only reported kindred with any *RIMS1* mutation, with significantly enhanced cognitive function in at least the verbal (likely to be related to general ability, g) and executive domains. *RIMS1* is an

excellent candidate gene to influence cognitive function. Rim1 α protein regulates synaptic-vesicle fusion, and interacts with several other active-zone molecules, including α -liprins, Munc 13-1, CAST, SNAP-25, synaptotagmin and 14-3-3, generating a protein scaffold in the presynaptic terminal.²⁷ Rim1 α greatly enhances neurotransmitter exocytosis in a Rab3-dependent manner.²⁸ Absence of Rim1 α protein leads to severe impairment of learning and memory.¹⁷

Could the *RIMS1* mutation be a chance finding, unrelated to the eye or cognitive phenotype? The intrafamilial distribution of cognitive measures argues that the detected mutation is most probably causative, especially as it segregates with both the eye phenotype (which becomes clinically symptomatic) and the cognitive enhancement. Co-mingling in each outbred generation of mutation-carrying and wild-type sibs each with respective enhanced or normal cognitive phenotype and the respective co-segregating impaired or normal visual phenotype renders extremely unlikely the possibility of an intrafamilial founder effect unrelated to the *RIMS1* mutation, as supported quantitatively by our modelling, beyond linkage analysis alone.

We have not demonstrated a molecular basis by which the R844H substitution in *RIMS1* could enhance cognition. The possibility remains that there is a mutation (or mutations) in another gene (or genes) in the region between markers D6S430 and D6S1625¹², which is (or are) responsible for both the retinal and cognitive phenotypes, or the retinal and cognitive phenotypes separately. Within this region, there are at least 17 other genes. Two of these, *IMPG1* and *MYO6*, were considered good candidates for the eye phenotype alongside *RIMS1* on the basis of function and pattern of expression, and were screened by Johnson *et al.*¹² *BAI3* was subsequently screened (Cottrill and Hunt, unpublished data): no mutations were found in any of these other three genes. Yet other genes, such as *KCNQ5*, where there is no evidence for retinal expression, have not been examined, and would not be candidates for the eye phenotype, but could be for the brain phenotype. However, we consider it more likely on the basis of the available evidence, including biological plausibility,^{14 15 17} that the R844H mutation in *RIMS1* explains both the eye phenotype and the cognitive phenotype, with a lower LOD score

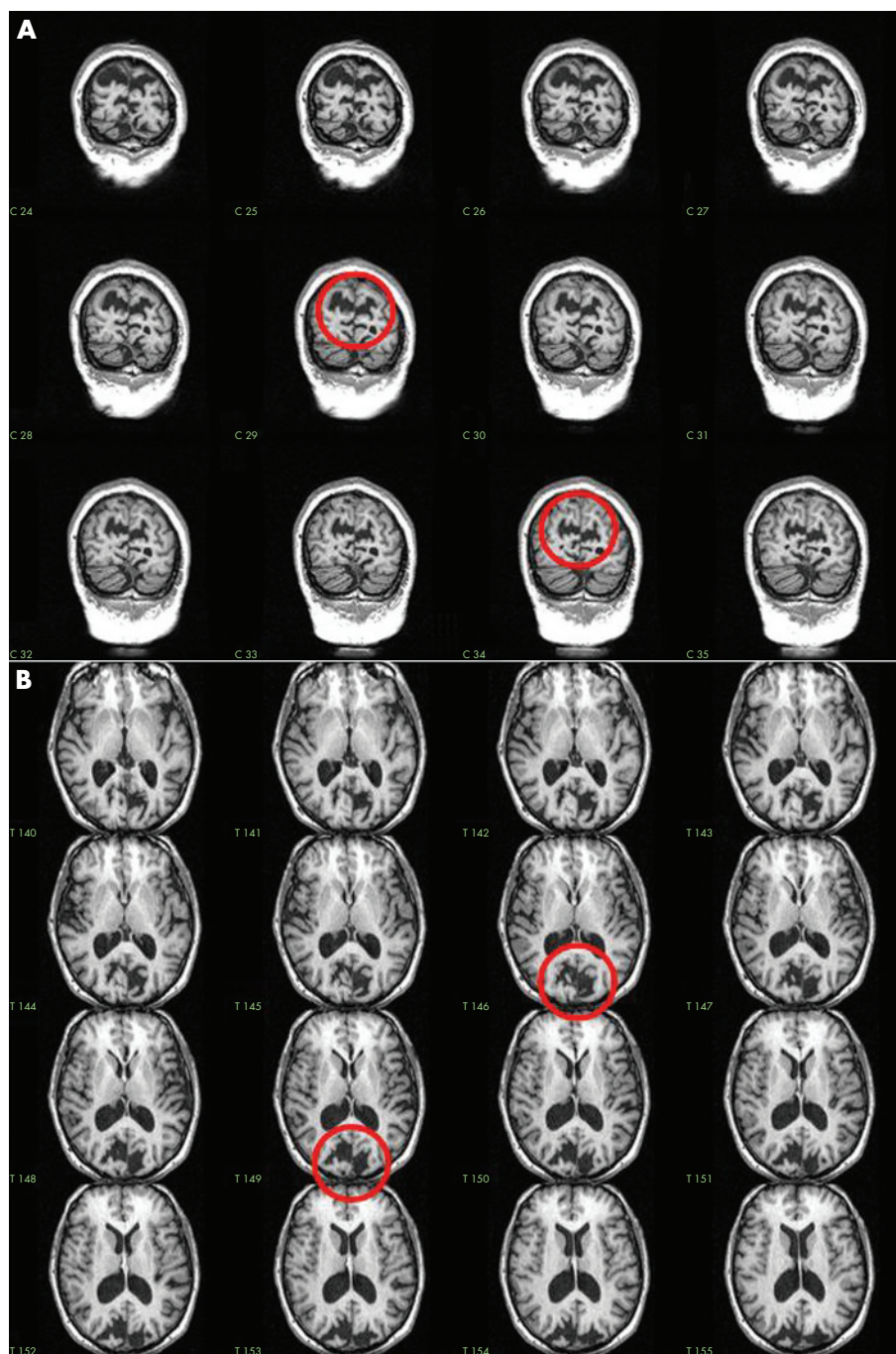


Figure 2 Serial reformatted T1-weighted magnetic resonance images from subject II:2, shown in (A) coronal and (B) axial planes. Note the widened cerebrospinal fluid spaces around an area of cortical malformation, shown encircled in selected images. Detailed inspection in three dimensions suggested that the malformation was most probably an area of polymicrogyria.

for the cognitive phenotype as this is more genetically complex and the mutation probably less penetrant.

One interpretation of our findings is that the *RIMS1* mutation in this kindred alone (ie, with this genetic background and environmental setting) is permissive of a gain of verbal cognitive abilities in response to impaired visual function, rather than being directly causative. This is inherently unlikely, especially in this kindred, as with increasing age higher cognitive abilities are usually associated with better, rather than defective, sensory processing.²³ In the *RIMS1* kindred, development of visual symptoms occurs later than the age at which values of cognitive measures (eg, NART, VIQ) are set—for example, subject III:2 did not develop symptomatic retinal

dystrophy until the age of 42 years, but had VIQ 146 at the age of 49 years. In addition, three mutation carriers in the kindred had normal visual function at the time of cognitive assessment. Additionally, visual impairment due to *PAX6* mutation in unrelated subjects with earlier and more severe loss of central vision was not associated with any gain in VIQ. Therefore, the data suggest that the observed genetic change is a gain-of-function mutation leading to increased performance in specific cognitive domains in affected humans, irrespective of other genetic background and unrelated to visual impairment. The *RIMS1* mutation studied may affect other cognitive domains—the paradigm we applied is biased to tests requiring only auditory presentation and verbal response, and verbal, not

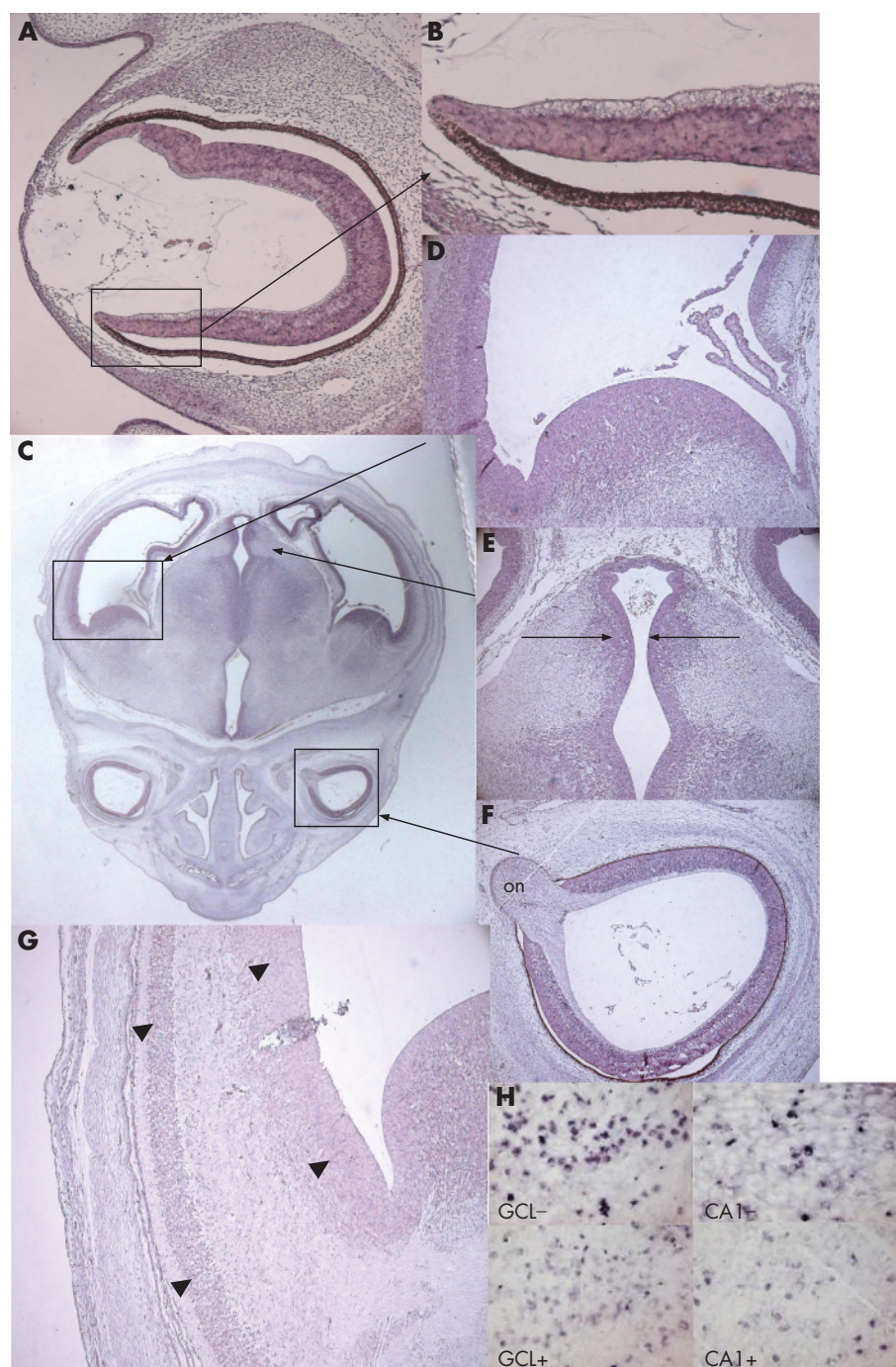


Figure 3 In situ hybridisation for *RIMS1* mRNA in human embryonic eye (A) from Carnegie stage 21. The purple labelling represents expression of *RIMS1*, found as expected in the developing retinal pigment epithelium, shown at higher power in (B). (A) and (B) are sagittal images. In (C), a tilted coronal section through the developing human head at fetal stage 1 shows expression in the developing cortical anlage (D, higher power), hippocampal anlage (E, arrows) and retina, but not in the optic nerve (on) (F). Expression is present in both the subventricular zone and the cortical plate (G, arrowheads). In adult human hippocampus (H), from a patient with temporal lobe epilepsy, expression (purple labelling) is seen in small cells in the (neuron-depleted) dentate granule cell (GCL-) layer and in the CA1 sector (CA1-), compared with sections labelled with the sense probe as control (GCL+, CA1+).

visual, processing, and was used for subjects we suspected of having cognitive impairment, not cognitive enhancement. Thus, only a limited cognitive assessment was undertaken, in line with our previous reports. We note that, undoubtedly, other genes must also contribute to cognitive scores in this kindred.

The *RIMS1* gene shows extensive organ-specific alternative mRNA splicing.¹² Thus, although the mutation lies in the protein C₂A domain, and is shared by the eye and brain isoforms, nevertheless the ocular neurophysiology in the kindred¹³ need not reflect mutant brain *RIMS1* protein function. Lack of homogeneity within a kindred for the functional consequences of gene mutation has previously been

noted.⁶ Our qualitative structural findings also implicate *RIMS1* as a candidate gene for bilateral parasagittal polymicrogyria. Brain malformation is not a prerequisite for the cognitive phenotype in this kindred, and, indeed, when cognition is altered in patients with polymicrogyria, it is usually impaired.

RIMS1 is a plausible candidate because of data from animal studies discussed earlier, and also in terms of the pattern of expression in humans. Thus, human *RIMS1* mRNA is detectable from an early age not only in retinal, but also in cerebral, anlage (fig 3) and adult human hippocampus (fig 3H). *RIMS1* protein is more abundant in phylogenetically newer brain regions than in older regions.²⁸ However, we were not able to demonstrate accelerated evolution of *RIMS1* in the primate

lineage, nor were genes encoding RIMS1-interacting molecules primate-fast outliers in a previous analysis.⁵

The population genetic data suggest that *RIMS1* may not have a major role in normal variation of the studied cognitive measures in a Western European population. Any evolutionary advantage of this particular mutation could be counterbalanced by the concomitant severe visual phenotype, albeit late onset. Although population genetic studies can provide useful, or even conclusive, evidence that a gene and its encoded protein have a biological function, negative population genetic studies do not exclude such a role. The genetics of Parkinson's disease illustrate this well: rare, early-onset Mendelian cases may be caused by mutation in *PINK1*,²⁹ but common variation in *PINK1* does not influence the risk of common, sporadic Parkinson's disease.³⁰ Further work is clearly required before a role for *RIMS1* in human cognition can be formally accepted. However, even if the observed *RIMS1* mutation turns out to be a private mutation enhancing cognition in affected members of this kindred only, the observation remains important because as the first such mutation identified in humans, it raises the possibility that genetic mutations may lead to increased, as well as reduced, cognitive function in man.

ACKNOWLEDGEMENTS

We thank the kindred. The work was supported by the Guide Dogs for the Blind Association (ATM, DMH), British Retinitis Pigmentosa Society (DMH, ATM), the UK's Medical Research Council UK (SLF) and the National Society for Epilepsy, UK (SMS, PJT). The Lothian Birth Cohort 1921 was supported by the UK's Biotechnology and Biological Sciences Research Council. IJD is the recipient of a Royal Society-Wolfson Research Merit Award. Human embryonic and fetal material was provided by the Medical Research Council/Wellcome Trust-funded Human Developmental Biology Resource.

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Competing interests: None declared.

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Supplementary material

Cognitive tests in kindred

Intellectual level

The Vocabulary, Digit Span, and Similarities subtests from the WAIS-R were administered and the scores were pro-rated to obtain an estimate of VIQ (ref 19, main text).

Executive Functions

1). Verbal fluency: The subject had to produce as many words beginning with the letter S (phonemic fluency) and as many animal names (semantic fluency) both in a minute. Performance on fluency tests, particularly for the phonemic category (letter “s”), is a sensitive indicator of frontal lobe functioning.³¹

2). The Hayling Test is a response suppression task. The subject has to complete two series of 15 sentences each missing the last word. In the first section a sensible completion is required and in the second a nonsensical completion. The test yields two measures of mental processing speed and an error score for the second series. Performance on this measure has been shown to involve frontal brain regions in healthy individuals³² and to be adversely affected by frontal lobe pathology.³³ 3) Cognitive estimates. This is a semantic reasoning task that requires the subject to provide a reasonable estimation to ten questions that have no exact answer, based on their available semantic knowledge. The questions are of the format ‘How fast do race horses gallop?’. Penalties are awarded for inaccurate responses and the higher the score the poorer the reasoning demonstrated. This test has been shown to be sensitive to frontal lobe pathology.^{19, 34}

Memory.

1). Verbal Recall. This was assessed using the Story Recall subtest from the Adult Memory and Information Processing Battery.³⁵ The subject is read a short story and then has to recall as many details as possible immediately following presentation and again following a delay of 30 minutes. Performance measures used were the immediate recall score and the % retained score (delayed recall/immediate recall x 100). 2). Verbal Learning. The List Learning test from the AMIPB was employed. The subject is presented with a list of 15 words on five occasions and following each presentation has to recall as many of the words as possible. A second list of words is then presented and following one attempt at recall is required to recall as many words from the first list (delayed recall). The total number of words remembered in the learning phase (verbal learning trails) and in the delayed recall condition (verbal learning delay) were recorded.

MRI in kindred

Coronal T1-weighted images were acquired at 1.5T: acquisition parameters were TE= 4.2, TI= 450, TR= 15, NEX= 1, flip angle= 20, acquisition matrix 256 x 128, field of view 24cm, producing 124 contiguous slices, voxel dimension 0.9375mm x 0.9375mm x 1.5mm. Data were reformatted in multiple planes to allow careful examination of regions of interest. Additionally T2 and FastFLAIR sequences were obtained (T2 and PD sequence: TE1= 30, TE2= 120, TR= 2000, NEX= 1, acquisition matrix 256 x 128, field of view 24x18cm, slice thickness 5mm contiguous; FastFLAIR sequence TE1= 152, TE2= 2200, TR= 10002, NEX= 1, acquisition matrix 256 x 128, field of view 24cm, slice thickness 5mm contiguous).

Cognitive testing in the LBC1921 cohort

Mini-Mental State Examination (MMSE): MMSE³⁶ was used as a screen for dementia. The maximum score is 30. A score of less than 24 was used as an indicator of possible dementia.

Moray House Test (MHT): This general mental ability test was previously described in detail.^{18, 37} It mainly assesses verbal reasoning skills. Subjects took this test at the age of 11 and again at about age 79.

Raven's Standard Progressive Matrices: Non-verbal reasoning was examined at age 79 using Raven's Standard Progressive Matrices.³⁸

Verbal fluency: Prefrontal executive function was examined at age 79 using the Verbal Fluency test. Subjects named as many words as possible in one minute for each of the letters C, F, L.^{39,40}

Logical Memory: Verbal declarative memory was measured at age 79 using the Logical Memory test, which is a sub-test from Wechsler Memory Scale-Revised.⁴¹

National Adult Reading Test (NART): NART was used at age 79 to assess prior cognitive ability.⁴²⁻⁴⁴

g factor: A g factor, as a measure of general intelligence, was created by principal component analysis of the age 79 MHT, Raven's Standard Progressive Matrices, Verbal Fluency and Logical Memory scores. A single component accounted for 53.5% of the total variance.

Genotypic analysis in LBC1921 cohort

Genotyping of the tagging SNPs was carried out by TaqMan fluorescence based allelic discrimination, with primers designed using the Applied Biosystems (ABI) Assay by Design tool (Applied Biosystems, Foster City, CA). Polymerase chain reactions were carried out according to the standard ABI protocol for 5µl reaction volume on 384-well plates. SDS version 2.1 software (Applied Biosystems, Foster City, CA) was used for analysis. Primer sequences are available on request.

Statistical analysis

Multiple regression analyses were implemented in STATISTICA (StatSoft Inc, Tulsa, USA), with the respective cognitive measure as the dependent variable, and genotype scores as the independent predictors. Genotypes were scored using an unrestricted model allowing arbitrary effects of the genotypes at each locus. Regression analyses were performed both with and without including sex as an independent variable. Haplotype analyses were implemented using the haplo.stats software⁴⁵, in the R console. Those individuals with data missing from more than two loci or present at less than two loci in the section were excluded from the haplotype analysis.

Association was undertaken in 469 individuals for the available cognitive measures that most clearly paralleled those enhanced in the *RIMS1* affected subjects: these were the National Adult Reading Test (NART), Wechsler Logical Memory, and verbal fluency.

Nineteen tagging SNPs were selected, giving average-locus haplotype r^2 values for the five sections within the gene of 0.81, 0.81, 0.81, 0.81 and 0.86, and an average-locus haplotype r^2 value of 0.82 within the gene as a whole. Average-locus r^2 values for the flanking 100kb sections were 0.89 and 0.67 for the upstream and downstream sections respectively. No significant violations of Hardy-Weinberg equilibrium were observed for any of the tagging SNPs. Each individual SNP was checked for association with the cognitive measures (Supplementary Table 2), including both sex

and genotype as predictors. There were no sex-by-genotype interactions, and none of the individual SNPs was associated with any of the cognitive measures. Sex was a significant predictor of score for Raven's matrices ($P=0.0005$), and g ($P=0.03$; P values uncorrected for multiple testing). Additionally, the haplotypes generated by the tagging SNPs from each section showed no association with any of the cognitive measures (Supplementary Table 3).

Generation of *RIMS1* riboprobes and in situ hybridisation.

The cDNA sequence (nucleotides 590-995 numbering from the start codon) was amplified using the polymerase chain reaction from human brain cDNA (Clontech) using KOD DNA polymerase (Novagen). This was A-tailed and then inserted into the vector pGEM-T Easy (Promega). The construct was then sequenced using vector primers to check on the orientation of the insert. Antisense and sense probes were generated by in vitro transcription using T7 and SP6 RNA polymerase under standard procedures. Digoxigenin-dUTP was incorporated into riboprobes during in vitro transcription by using the DIG RNA labelling mix (Roche) according to the manufacturer's instructions. In situ hybridization was performed as reported in Lai et al.⁴⁶

***RIMS1* evolution analysis results**

Preliminary Analysis – Human-Chimp and Rat-Mouse Alignment:

Primate Lineage:

Nonsynonymous Changes = 4

Synonymous Changes = 10

$$\begin{aligned} K_a &= \text{NS changes/nonsynonymous site} \\ &= 4 / 3637.83 = 0.0010 \end{aligned}$$

$$\begin{aligned} K_s &= \text{S changes/synonymous site} \\ &= 10 / 1383.17 = 0.0072 \end{aligned}$$

$$K_a/K_s = .0010/.0072 = 0.152$$

Rodent Lineage:

Nonsynonymous Changes = 18

Synonymous Changes = 138

$$\begin{aligned} K_a &= \text{nonsynonymous change/nonsynonymous site} \\ &= 18 / 3002.67 = 0.0060 \end{aligned}$$

$$\begin{aligned} K_s &= \text{synonymous change/synonymous site} \\ &= 138 / 1116.33 = 0.124 \end{aligned}$$

$$K_a/K_s = 0.0060/0.124 = 0.0484$$

Statistical Significance:

Dorus et al.⁵ tested for significance with a one-tailed Fisher's exact test using the numbers of nonsynonymous and synonymous substitutions as the text values. These values are too large, therefore Chi-squared is more appropriate.

	Rodent	Primate	
Nonsynonymous	18	4	$X^2 = 3.32$
<i>Synonymous</i>	<i>138</i>	<i>10</i>	$P = 0.065$

There is a marginally significant disparity between substitution rates in primates and rodents. However, the Human-Chimp alignment is expected to reduce statistical power in detecting evolutionary signatures due to the sequence similarities.

Human-Macaque and Rat-Mouse Alignment:
Primate Lineage:

Nonsynonymous Changes = 11
Synonymous Changes = 28

K_a = NS changes/nonsynonymous site
= 14 / 3563.3 = 0.0039
 K_s = S changes/synonymous site
= 52 / 1353.67 = 0.038

$K_a/K_s = .0039/.038 = 0.102$

Rodent Lineage:

As Above:

$K_a/K_s = 0.0060/0.124 = 0.048$

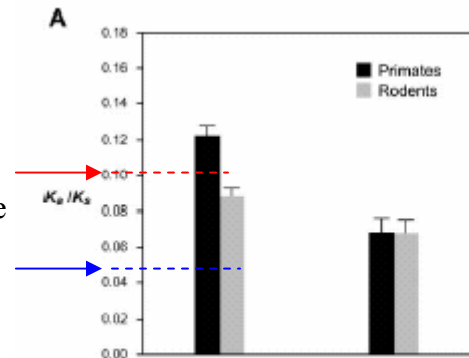


Figure 4: Placement of Primate (red) and Rodent (blue) K_a/K_s values for RIM-1 with respect to the average K_a/K_s obtained for 214 CNS genes.

Statistical Significance:

	Rodent	Primate	
Nonsynonymous	18	14	$X^2 = 3.54$
<i>Synonymous</i>	<i>138</i>	<i>52</i>	$0.5 \leq P \leq 0.1$

K_a/K_s for Human-Macaque is slightly less significant than that of Human-Chimp.
The summary of all sequences and K_a/K_s calculations is in the Tables below, A-C.

Table A: Number of Sequence Changes Between Species:

	Non-Synonymous	Synonymous	Non-Synonymous	Synonymous	Non-Synonymous	Synonymous
	Chimpanzee		Macaque		Squirrel Monkey	
Human	4	10	14	52	22	43
Chimp	----		18	57	25	49
Macaque	----		----		19	48

Table B: Chi Square Values of each primate alignment compared to the Rat-Mouse alignment

	Chimpanzee	Macaque	Squirrel Monkey
Human	3.31	3.52	15.40*
Chimpanzee		5.98*	16.34*
Macaque			9.58*

Chi Square tests of all primate comparisons vs. the rodent comparison show significance (*) in human lineage only when compared with the squirrel monkey (significance = $P > 3.84$). This comparison does not represent a similar divergence time (as the Human-Macaque relationship does) and therefore is probably not as significant as it appears. The Chimp-Macaque Ka/Ks is significantly different from the rodent Ka/Ks, however.

Table C: Pairwise Primate Ka/Ks Calculations.

	Chimpanzee	Macaque	Squirrel Monkey
Human	0.151919821	0.10227747	0.19195082
Chimpanzee	--	0.120054536	0.191678276
Macaque	--	--	0.14858768

Ka/Ks values based on the alignments of species gene sequences.

Figure: Identification of Inter-species Sequence Changes in the Phylogenetic Tree

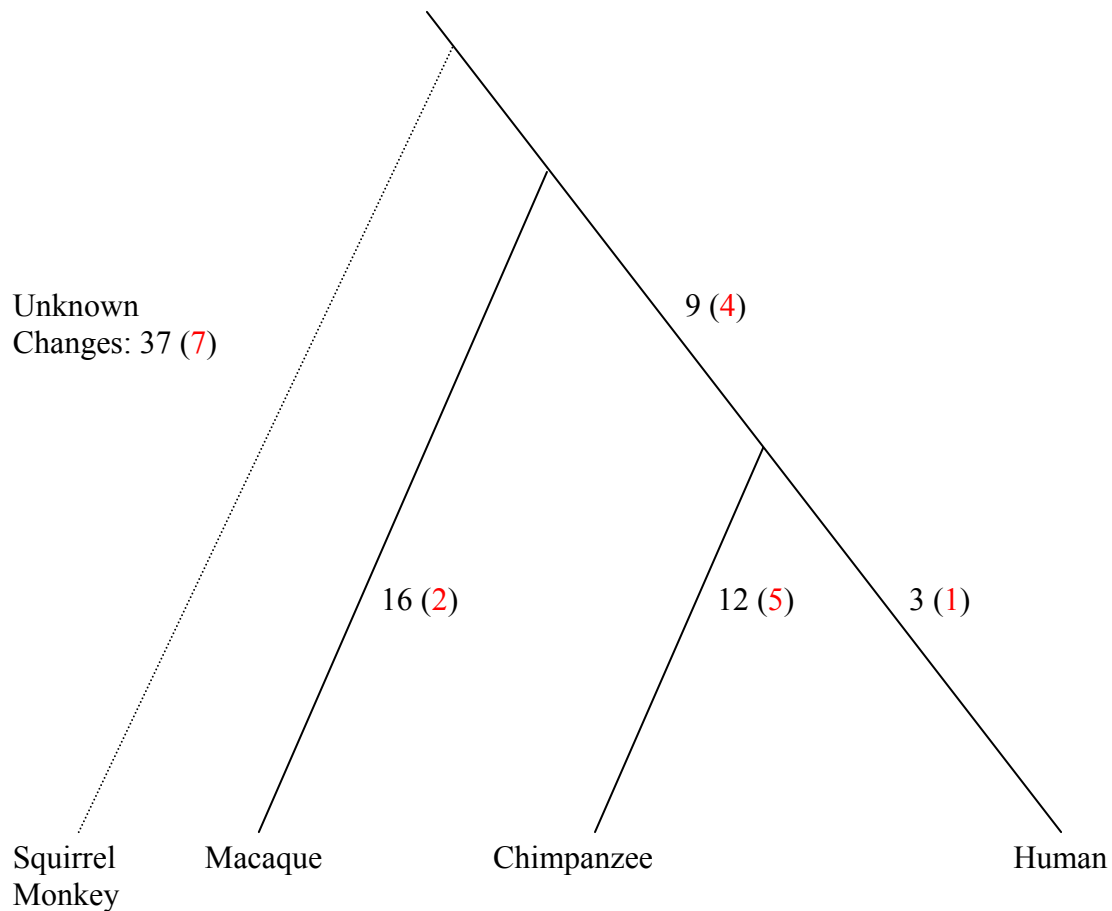


Figure. Sequence analysis using the squirrel monkey as an outlying reference allows the identification of the origin of sequence changes between species (nonsynonymous changes in red). Of a total 77 variants, only three of them are specific to the human lineage (two synonymous changes and one nonsynonymous). The chimpanzee has the most species-specific nonsynonymous changes (5), and the human-chimpanzee common ancestor has four. Due to limitations in the sequencing of squirrel monkey exons, 37 variants were unable to be assigned (seven of which are nonsynonymous changes, the majority of which (22) are located in exon 7, an exon that does not lie in a functional domain).

Supplementary Material References

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Supplementary Table 1. Visual acuities in *RIMS1* and *PAX6* cohorts

	Visual acuity, right eye	Visual acuity, left eye
<i>RIMS1</i> affected individuals		
II:2	6/60	6/60
II:6	3/36	3/60
II:8	6/9	6/9
III:1	6/36	6/36
III:2	6/9	6/9
III:5	3/60	6/60
IV:2	6/6	6/6
IV:3	6/18	3/60
<i>PAX6</i> affected individuals*		
	6/36	6/24
	no perception of light	6/60
	n/a	n/a
	6/36	6/60
	6/60	6/60
	6/36	6/36
	no perception of light	no perception of light
	hand movements only	hand movements only
	3/60	3/60
	2/60	2/60
	1/60	1/60
	5/60	5/60
	3/60	3/60
	3/60	no perception of light

* Individuals from Thompson et al., 2004 (ref 7).

Supplementary Table 2: Association of individual tagging SNPs with cognitive measures. P values are shown both with and without sex factored into the analysis. Thick black lines depict sections the gene was divided into on the basis of pairwise D'.

***genotypes not significant predictors: there is no interaction (*sex by genotype*)**

	sex	rs2183066	rs4235866	rs4707954	rs1028387	rs10942989	rs1482567	rs1564609
g	0.03	0.23	0.51	0.36	0.11	0.08	0.55	0.27
g with sex	-	0.07	0.11	0.06	0.06	0.02	0.09	0.06
NART	0.55	0.17	0.5	0.75	0.61	0.83	0.02	0.08
NART with sex	-	0.3	0.63	0.89	0.78	0.92	0.06	0.16
Logical memory	0.13	0.96	0.4	0.79	0.87	0.84	0.35	0.47
Logical memory with sex	-	0.6	0.25	0.43	0.47	0.69	0.21	0.23
IQ 11	0.36	0.13	0.1	0.81	0.08	0.8	0.12	0.24
IQ 11 and sex	-	0.23	0.14	0.69	0.1	0.81	0.2	0.4
IQ 79	0.05	0.1	0.33	0.62	0.25	0.21	0.3	0.45
IQ 79 and sex	-	0.02	0.11	0.04	0.16	0.04	0.03	0.06
Raven's Matrices	0.0005	0.35	0.7	0.65	0.32	0.14	0.1	0.13
Raven's Matrices and sex	-	0.006*	0.005*	0.005*	0.007*	0.002*	0.0008*	0.003*
Verbal fluency	0.54	0.09	0.71	0.88	0.45	0.08	0.24	0.91
Verbal fluency and sex	-	0.13	0.75	0.88	0.64	0.14	0.2	0.92

	rs9360524	rs1482574	rs1482574	rs11756248	rs2496517	rs2697433	rs4256398	rs9442769
g	0.93	0.93	0.93	0.26	0.07	0.18	0.95	0.32
g with sex	0.39	0.31	0.31	0.14	0.07	0.04	0.21	0.09
NART	0.99	0.33	0.33	0.08	0.09	0.2	0.12	0.24
NART								
with sex	0.96	0.46	0.46	0.17	0.17	0.27	0.19	0.39
Logical								
memory	0.32	0.94	0.94	0.07	0.02	0.15	0.67	0.22
Logical								
memory								
with sex	0.44	0.54	0.54	0.13	0.03	0.15	0.63	0.19
IQ 11	0.83	0.63	0.63	0.99	0.96	0.8	0.92	0.81
IQ 11 and								
sex	0.93	0.64	0.64	0.88	0.97	0.92	0.94	0.9
IQ 79	0.58	0.79	0.79	0.04	0.06	0.27	0.5	0.04
IQ 79 and								
sex	0.4	0.27	0.27	0.03	0.02	0.02	0.06	0.1
Raven's								
Matrices	0.61	0.75	0.75	0.17	0.06	0.03	0.61	0.17
Raven's								
Matrices								
and sex	0.03*	0.01*	0.01*	0.008*	0.006*	0.0006*	0.008*	0.004*
Verbal								
fluency	0.54	0.98	0.98	0.21	0.67	0.02	0.57	0.17
Verbal								
fluency								
and sex	0.55	0.85	0.85	0.33	0.6	0.05	0.73	0.18

	rs9342944	rs1416546	rs1361311	rs2815715	rs9351921
g	0.63	0.47	0.6	0.5	0.56
g with sex	0.21	0.1	0.19	0.14	0.12
NART	0.98	0.53	0.89	0.28	0.53
NART					
with sex	0.89	0.66	0.96	0.37	0.63
Logical					
memory	0.19	0.24	0.52	0.41	0.54
Logical					
memory					
with sex	0.17	0.14	0.45	0.25	0.39
IQ 11	0.16	0.33	0.39	0.7	0.43
IQ 11 and					
sex	0.19	0.37	0.56	0.59	0.6
IQ 79	0.53	0.89	0.61	0.44	0.16
IQ 79 and					
sex	0.28	0.21	0.05	0.1	0.02
Raven's					
Matrices	0.94	0.84	0.7	0.91	0.21
Raven's					
Matrices					
and sex	0.01*	0.002*	0.007*	0.01*	0.001*
Verbal					
fluency	0.13	0.56	0.28	0.07	0.88
Verbal					
fluency					
and sex	0.4	0.78	0.36	0.09	0.96

Supplementary Table 3. Haplotype association statistics.

Scores shown are the P-values for the global score statistic calculated for the overall haplotype effect for each gene section (labelled by dbSNP rs number) for each cognitive measure. P values are uncorrected for multiple testing.

	rs10498879- rs2040055	rs9342903- rs1564609	rs7743295- rs1482574	rs12213714- rs2496531	rs1015946- rs2807530	rs2746200- rs10943011	rs2815736- rs9446692
g	0.37 (0.38)	0.45 (0.45)	0.29 (0.31)	0.27 (0.27)	0.91 (0.91)	0.30 (0.34)	0.70 (0.74)
NART	0.34 (0.37)	0.45 (0.47)	0.15 (0.12)	0.04 (0.03)	0.70 (0.72)	0.47 (0.51)	0.28 (0.29)
Logical memory	0.46 (0.48)	0.92 (0.89)	0.91 (0.91)	0.31 (0.31)	0.68 (0.67)	0.21 (0.21)	0.30 (0.35)
Verbal Fluency							
Total	0.22 (0.23)	0.20 (0.20)	0.78 (0.77)	0.86 (0.85)	0.86 (0.86)	0.22 (0.19)	0.02 (0.05)
IQ 11	0.98 (0.98)	0.26 (0.28)	0.36 (0.39)	0.21 (0.21)	0.98 (0.97)	0.94 (0.94)	0.17 (0.23)
IQ 79	0.16 (0.16)	0.30 (0.30)	0.16 (0.14)	0.09 (0.09)	0.52 (0.53)	0.14 (0.15)	0.85 (0.82)
Raven's Matrices	0.51 (0.53)	0.38 (0.37)	0.07 (0.06)	0.36 (0.34)	0.77 (0.78)	0.38 (0.36)	0.57 (0.61)

(scores in parenthesis are global simulation P values)